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Medium-Chain Triglycerides in Combination with Leucine and Vitamin D Benefit Cognition in Frail Elderly Adults: A Randomized Controlled Trial

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Summary The combined supplementation of medium-chain triglycerides (MCTs), L-leucine-rich amino acids, and cholecalciferol (vitamin D_3) increase muscle strength and function in frail elderly individuals. However, their effects on cognition are unknown. We enrolled 38 elderly nursing home residents (mean age±SD, 86.6±4.8 y) in a 3-mo randomized, controlled, parallel group trial. The participants were randomly allocated to 3 groups: the first group received a L-leucine (1.2 g)- and cholecalciferol (20 μg)-enriched supplement with 6 g of MCT (LD+MCT); the second group received the same supplement with 6 g of long-chain triglycerides (LD+LCT); and the third group did not receive any supplements (control). Cognition was assessed at baseline and after the 3-mo intervention. The difference in changes among the groups was assessed with ANCOVA, adjusting for age and the baseline value as covariates. After 3 mo, the Mini-Mental State Examination (MMSE) score in the LD+MCT group increased by 10.6% (from 16.6 to 18.4 points, $p<0.05$). After 3 mo, the Nishimura geriatric rating scale for mental status (NM scale) score in the LD+MCT group increased by 30.6% (from 24.6 to 32.2 points, $p<0.001$), whereas that in the LD+LCT and control groups decreased by 11.2% (from 31.2 to 27.7 points, $p<0.05$) and 26.1% (from 27.2 to 20.1 points, $p<0.001$), respectively. The combined supplementation of MCTs (6 g), L-leucine-rich amino acids, and cholecalciferol may improve cognitive function in frail elderly individuals.

Key Words MCT, vitamin D, leucine, cognition, Alzheimer's disease

Cognitive impairment is common in elderly individuals (*1*) and has emerged as a serious public health concern, placing an immense burden on the individual, family, community, and health care resources. The cause of cognitive impairment is primarily Alzheimer's disease (AD) (*2*). Although there is no cure for AD, non-pharmacologic interventions and medications that modulate neurotransmission can slow symptomatic progression (*3*). A combination of interventions, such as non-pharmacologic treatments (supplements and exercise), and pharmacotherapy, with complementary mechanisms of action, may provide a rational approach that may result in the preservation of cognitive function in patients with AD (*3*).

Previously, in a search for nutritional supplements that frail elderly individuals can easily eat and which would preserve their muscle strength and function, we found that the combined supplementation of medium-chain triglycerides (MCTs) (6 g), leucine-rich amino acids, and cholecalciferol (vitamin D_3) at dinner significantly improved muscle strength and function in these individuals in a randomized controlled trial (RCT) (*4*). During this trial, we also examined the changes in cognitive function. Here, we report that cognitive functions were substantially improved by the combined supplementation of small amounts of MCTs (6 g), leucine-enriched essential amino acids (EAAs) (40% leucine in 3 g EAAs), and cholecalciferol (20 μg, 800 IU).

MATERIALS AND METHODS

Participants. Initial interest in this study was targeted toward all participants who resided in a nursing home (n=65) (Fig. 1). However, 25 participants were excluded on the basis of the criteria, and 2 participants were moved to other facilities before the initiation of the dietary intervention, resulting in a total of 38 participants (11 men and 27 women with a mean age of 86.6±4.8 [±SD] y) (*4*). The exclusion criteria were body mass index (BMI) of >23 kg/m^2 (to avoid a further increase in body weight); <65 y of age; receiving parenteral nutrition; difficulty in swallowing; severe heart failure, lung, liver, kidney, or blood disease; fasting blood glucose level of ≥200 mg/dL; blood creatinine level of ≥1.5 mg/dL; CRP level of ≥2.0 mg/dL; or allergy to the supplements used in the study.

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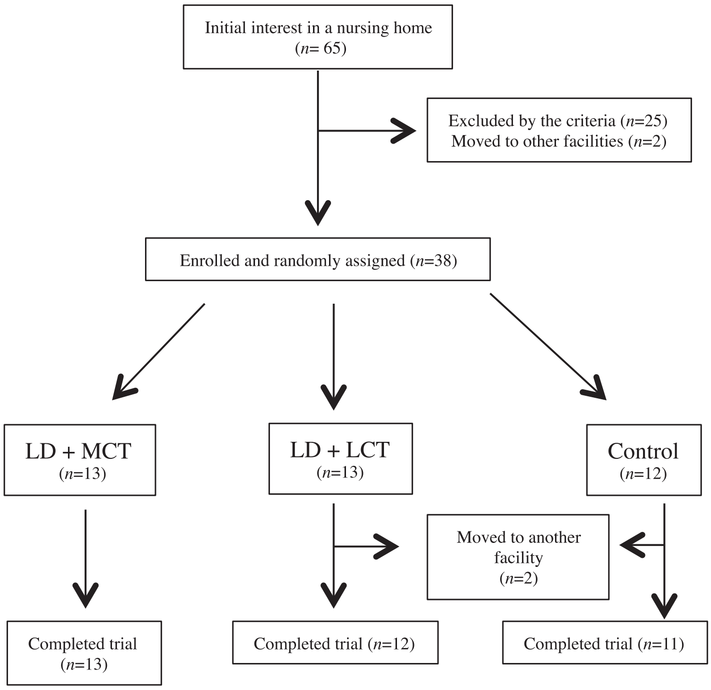

Fig. 1. Flowchart of the 3-mo randomized, controlled, single-blinded, parallel group trial. LCTs, long-chain triglycerides; LD, leucine- and cholecalciferol-enriched supplement; MCTs, medium-chain triglycerides.

The present study was approved by the Human Ethics Committee of Showa Women's University (No. 14-10) on July 18, 2014. Participant recruitment was conducted from August 24, 2014 to September 3, 2014. The participants and their family members were informed of the nature of the experimental procedures before their written informed consent was obtained. The intervention took place from September 17, 2014 to December 15, 2014 at the Day Care SKY facility in Yokohama, Japan. The procedures were in accordance with either the ethical standards of the institutional committee on human experimentation or the Helsinki Declaration of 1975 (as revised in 1983). This trial was registered at the University Hospital Medical Information Network Clinical Trials Registry as UMIN000017567.

Study design. We performed a 3-mo randomized, controlled, single-blinded, parallel group trial in which the 38 participants were randomly allocated into three groups as described previously (Fig. 1) (*4*). Sealed envelopes containing the written informed consent of the individual participants (or their family members) were thoroughly shuffled. Thirteen participants (envelopes) each were allocated to the first and second groups, and 12 participants were allocated to the third group. Allocation was conducted by a person who was not a member of this study.

The first group (n=13; men, n=4; women, n=9; mean age, 85.5±3.7 y) received a leucine- and cholecalciferol-enriched supplement with MCTs (LD+MCT). As an energy-matched control to the supplementation of MCTs, the second group (n=13; men, n=4; women, n=9; mean age, 87.7±3.9 y) received the same leucine- and cholecalciferol-enriched supplement with long-chain triglycerides (LCTs) (LD+LCT). A third group (n=12; men, n=3; women, n=9; mean age, 86.8±6.5 y) did not receive any supplements (control).

The participants' cognitive performance was assessed at baseline and after the 3-mo intervention. Food records for the individual participants were collected every day for 3 mo during the baseline period and for an additional 3 mo during the intervention period (*4*). Exercise protocols that were conducted in the nursing home were not changed between the baseline and intervention periods.

Blinding. The tube containing the supplement was visible to the participants, and thus, the participants in the intervention and control groups could not be blinded. However, the participants in the intervention groups were blinded in the sense that they could not distinguish between the supplements in the LD+MCT and LD+LCT groups.

The examiners who oversaw the Mini-Mental State Examination (MMSE) and Nishimura geriatric rating scale for mental status (NM scale) tests were unaware of the participants' groups.

Study products. The leucine- and cholecalciferol-enriched supplement (Amino care jelly Leucine 40 containing Amino L40) was purchased from Ajinomoto Co., Inc. (Tokyo, Japan). One tube (100 g, 30 kcal) of Amino care jelly Leucine 40 was given at the beginning of dinner. One tube contained EAAs (3 g) [leucine (1.2 g), isoleucine (0.3 g), valine (0.3 g), other amino acids (1.2 g)], carbohydrate (9.7 g), sodium (75 mg), cholecalciferol (20 μg, 800 IU), thiamin (0.2 mg), pyridoxine (0.2 mg), cyanocobalamin (0.4 μg), and water (87 g). The detailed contents of the tube were described previously (*4*). The leucine and vitamin D supplement contains 30 kcal yet contains 9.7 g carbohydrate and 3.0 g of amino acids because carbohydrate may contain

Table 1. MMSE and NM scale score at baseline and after the 3-mo intervention and their changes in the LD+MCT, LD+LCT, and control (no intervention) groups.

Measure and Group	*n*	Baseline	3 mo	Change	% Change	*p* value
MMSE						
Total score (total 30 points)						0.017
LD+MCT	13	16.6±6.8	18.4±7.0*	1.8±2.9[a]	10.6	
LD+LCT	12	18.4±9.2	18.4±10.1	0.0±2.7[ab]	0.0	
Control	11	16.3±10.3	14.9±10.0	−1.4±1.8[b]	−8.4	
Orientation (total 10 points)						0.79
LD+MCT	13	4.3±2.7	4.5±2.9	0.2±1.8	3.5	
LD+LCT	12	5.7±3.9	5.8±4.2	0.1±1.7	1.4	
Control	11	5.0±3.9	4.7±3.9	−0.3±0.9	−5.4	
Registration (total 3 points)						0.13
LD+MCT	13	2.6±1.0	3.0±0.0	0.4±1.0	14.5	
LD+LCT	12	2.6±1.0	2.6±0.9	0.0±0.4	0.0	
Control	11	2.6±0.9	2.2±1.4	−0.4±0.8	−14.5	
Attention and calculation (total 5 points)						0.22
LD+MCT	13	1.7±1.6	2.0±1.8	0.3±1.8	18.3	
LD+LCT	12	1.6±1.6	1.8±1.7	0.3±0.8	15.8	
Control	11	1.6±2.1	1.3±1.7	−0.4±0.7	−22.6	
Recall (total 3 points)						0.31
LD+MCT	13	1.3±1.3	1.5±1.3	0.2±0.6	17.6	
LD+LCT	12	1.8±1.4	1.7±1.4	−0.2±0.7	−8.7	
Control	11	1.0±1.3	1.0±1.3	0.0±0.4	0.0	
Language (total 9 points)						0.09
LD+MCT	13	6.7±1.9	7.4±1.8	0.7±1.0	10.3	
LD+LCT	12	6.8±2.6	6.6±2.9	−0.2±1.5	−2.5	
Control	11	6.0±3.7	5.7±3.4	−0.3±1.3	−4.5	
NM scale						
Total score (total 50 points)						<0.001
LD+MCT	13	24.6±9.3	32.2±9.3***	7.5±3.8[a]	30.6	
LD+LCT	12	31.2±14.5	27.7±13.7*	−3.5±6.3[b]	−11.2	
Control	11	27.2±14.2	20.1±14.1***	−7.1±4.8[b]	−26.1	
Housework, arranging of personal belonging (total 10 points)						<0.001
LD+MCT	13	3.9±2.3	4.9±2.2**	0.9±1.3[a]	23.5	
LD+LCT	12	4.6±3.0	4.0±2.8	−0.6±0.9[b]	−12.7	
Control	11	4.2±3.2	3.2±2.7**	−1.0±1.0[b]	−23.9	
Interest, volition, social relations (total 10 points)						<0.001
LD+MCT	13	4.4±1.9	5.9±2.1***	1.5±1.2[a]	35.1	
LD+LCT	12	5.9±2.9	4.9±3.0*	−0.5±1.7[b]	−16.9	
Control	11	4.9±3.4	3.6±3.1**	−1.3±1.6[b]	−25.9	
Conversation (total 10 points)						<0.001
LD+MCT	13	4.9±2.1	7.1±2.1***	2.2±1.6[a]	46.0	
LD+LCT	12	7.1±2.8	5.8±3.0**	−0.9±1.6[b]	−18.8	
Control	11	5.0±3.1	3.9±2.9*	−1.1±1.4[b]	−21.8	
Memory (total 10 points)						<0.001
LD+MCT	13	4.7±2.4	6.7±2.0***	2.0±1.6[a]	42.6	
LD+LCT	12	7.1±3.4	6.0±3.3*	−0.8±2.0[b]	−15.3	
Control	11	5.8±3.1	4.2±3.5***	−1.6±1.4[b]	−28.1	
Orientation (total 10 points)						0.001
LD+MCT	13	6.8±2.1	7.6±2.0	0.8±1.3[a]	12.5	
LD+LCT	12	7.7±2.4	6.6±3.2*	−0.8±2.0[ab]	−14.1	
Control	11	7.3±2.7	5.2±2.8***	−2.1±1.8[b]	−28.8	

Values are means±SD. Means of the changes in a column without a common letter differ, $p<0.05$. Different from baseline by Bonferroni correction test, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

p value represent the differences in the changes of variables among groups assessed by 1-factor ANCOVA adjusted by age and baseline values or by the Kruskal-Wallis test. The effect on "registration" and "attention and calculation" in MMSE were examined by the Kruskal-Wallis test, others were by 1-factor ANCOVA.

LD+MCT, leucine- and cholecalciferol-enriched supplement with 6 g of medium-chain triglycerides; LD+LCT, leucine- and cholecalciferol-enriched supplement with 6 g of long-chain triglycerides; MMSE, Mini-Mental State Examination; NM scale, Nishimura geriatric rating scale for mental status.

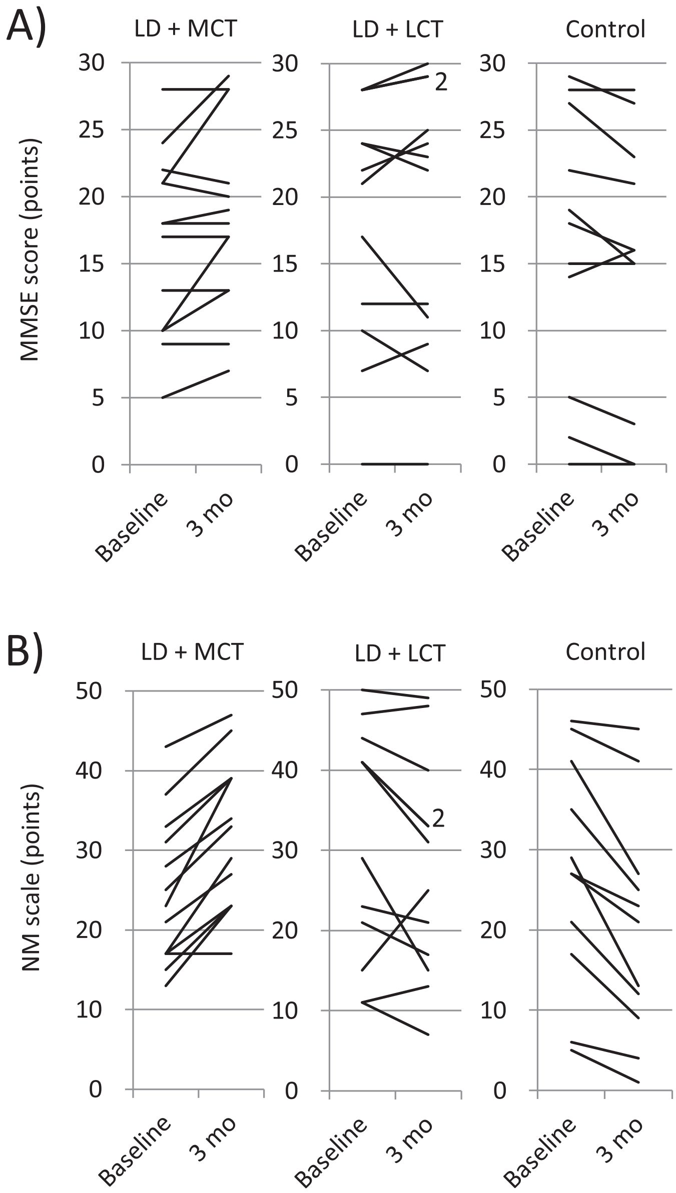

Fig. 2. Changes after the 3-mo intervention in MMSE points (A) and NM scale points (B) of the individuals in the LD+MCT, LD+LCT, and control groups. "2" indicates that two participants are shown on the same line. LD+MCT, a leucine- and cholecalciferol-enriched supplement with 6 g of medium-chain triglycerides; LD+LCT, a leucine- and cholecalciferol-enriched supplement with 6 g of long-chain triglycerides; MMSE, Mini-Mental State Examination; NM scale, Nishimura geriatric rating scale for mental status.

energy-less carbohydrate such as dietary fiber and resistant starch. However, the supplier (Ajinomoto Co., Inc.) did not disclose detailed information about the carbohydrate composition.

The MCTs (75% 8:0 and 25% 10:0 from total fatty acids) and LCTs (64% 18:1, 19% 18:2, and 9% 18:3 from total fatty acids) were kindly provided by the Nisshin OilliO Group Ltd. (Kanagawa, Japan). The detailed fatty acid compositions were also described previously (4). Six grams of MCTs (50 kcal; 8.3 kcal/g) or LCTs (54 kcal; 9 kcal/g) per day was mixed with foods such as steamed rice or miso soup at dinnertime. In total, the participants in the intervention groups increased their energy intake by about 80 kcal/d in comparison to the control group.

Cognitive function. To estimate cognitive function in the elderly individuals, two different tests were conducted: the MMSE, in which the participants answer questions themselves, and the NM scale, in which a caregiver or observer answers questions about the participant.

The MMSE is used worldwide, primarily for dementia screening (5). The MMSE (total 30 points) measures several cognitive areas, including orientation (10 points), registration (3 points), attention and calculation (5 points), recall (3 points), and language (9 points) (6). The participants whose recorded MMSE score was zero at baseline (1 participant in the LD+LCT group and 1 participant in the control group) were included in the analysis because they could communicate with others

Table 2. Correlation coefficients between changes in cognition test scores and changes in muscle mass, strength, and function in the LD+MCT group.

	n	Pearson correlation coefficient	
		MMSE	NM scale
Calculated right AMA	13	0.23 (p=0.46)	−0.14 (p=0.65)
Calculated left AMA	13	−0.39 (p=0.19)	−0.37 (p=0.21)
Right CC	12	0.24 (p=0.46)	−0.19 (p=0.55)
Left CC	12	0.23 (p=0.48)	−0.02 (p=0.95)
Right hand grip strength	13	−0.29 (p=0.33)	−0.29 (p=0.33)
Left hand grip strength	13	0.42 (p=0.15)	0.71 (p=0.01)
Right knee extension time	13	−0.06 (p=0.85)	−0.12 (p=0.69)
Left knee extension time	13	0.01 (p=0.98)	0.11 (p=0.71)
Walking speed	9	−0.03 (p=0.93)	0.15 (p=0.69)
Leg open and close test	13	0.32 (p=0.29)	−0.12 (p=0.70)
Peak expiratory flow	9	−0.06 (p=0.88)	−0.03 (p=0.94)

Notes: Results of Pearson correlation coefficients (and associated p values) are shown. Missing data (data that could not be collected at baseline and/or after intervention due to difficulty in performing tests) were not included in the analyses (*4*). LD+MCT, leucine- and cholecalciferol-enriched supplement with 6 g of medium-chain triglycerides; MMSE, Mini-Mental State Examination; NM scale, Nishimura geriatric rating scale for mental status; AMA, mid-upper-arm muscle area; CC, calf circumference.

by talking and were expected to increase their point scores by the intervention.

The NM scale was developed in Japan as a simple behavioral rating scale for mental state assessment (*7*). The NM scale (total 50 points) measures abilities in daily life, including housekeeping/arrangement of personal belongings (10 points), interest/volition/social relations (10 points), conversation (10 points), memory (10 points), and orientation (10 points) (*7*). In both tests, a higher score indicates better cognitive function.

The numbers of examiners who conducted the MMSE and NM scale were 1 and 3, respectively. The average score of 3 examiners in the NM scale was used.

Statistical analysis. All data are expressed as the mean±SD. The difference in changes (change value=3-mo intervention value−baseline value) among the groups was assessed with analysis of covariance (ANCOVA), adjusting for age and the baseline value as covariates. When an ANCOVA was significant, a Bonferroni correction test (a post-hoc test) was performed to compare the changes between groups and the baseline value with the intervention value within groups. Data on the changes were tested for the normality of distribution with the Kolmogorov-Smirnov test and for variations with Levene's test. When the variances were not homogeneous, the differences in the change among the groups were assessed by a non-parametric Kruskal-Wallis test. The Kruskal-Wallis test was used for data on "registration" and "attention and calculation" in the MMSE; however, they were not significant, and therefore post-hoc tests were not performed. The percentage of relative change (% change) was calculated as follows: % change=(mean of the 3-mo intervention value−mean of the baseline value)/mean of the baseline value · 100 (*8*). This value was then used to describe the degree of effect.

Pearson correlation coefficients were used to investigate associations between changes in cognition test scores and changes in muscle mass, strength, and function. Changes in muscle mass, strength, and function were shown in our previous report (*4*).

All statistical analyses were performed using the SPSS 20.0 software program (IBM, Chicago, IL). An α-level of 0.05 was used to determine statistical significance.

RESULTS

Participants and compliance

We enrolled 38 participants in the trial. Two men dropped out during the study: one from the control group was moved to another nursing home that was suitable for end-of-life care, and the other from the LD+LCT group was moved to a hospital for treatment of dysphagia and aspiration pneumonia (*4*). Thus, the total number of participants became 36. No differences in changes among the groups were found with regard to the daily intake of energy, protein, fat, carbohydrate, sodium, or vitamins (excluding supplements and oils) (*4*). Compliance with the supplements was 100%, and no side effects, including diarrhea, were reported. One participant from the LD+LCT group developed aspiration pneumonia during the intervention period and was moved to a hospital. It is unlikely that this was due to the supplements. It is possible that it occurred due to dysphagia.

MMSE

The differences in changes among groups in total score for the MMSE scale were significant (p=0.017) (Table 1). The increases in the MMSE score were greater in the LD+MCT group than in the control group (p<0.05). After the 3-mo intervention, the MMSE score in the LD+MCT group increased by 10.6% (from 16.6 to 18.4 points, p<0.05).

NM scale

The differences in changes among groups in total score for the NM scale were also significant ($p<0.001$) (Table 1). The increase in the NM scale score was greater in the LD+MCT group than in the LD+LCT and control groups ($p<0.05$). After the 3-mo intervention, the NM scale score in the LD+MCT group increased by 30.6% (from 24.6 to 32.2 points, $p<0.001$), whereas those in the LD+LCT and control groups decreased by 11.2% (from 31.2 to 27.7 points, $p<0.05$) and 26.1% (from 27.2 to 20.1 points, $p<0.001$).

The differences in changes among groups in scores of all five domains for the NM scale were also significant ($p\leq0.001$). The increases in scores of "housework, arrangement of personal belongings," "interest, volition, social relations," "conversation," and "memory" domains were greater in the LD+MCT group than in the LD+LCT group ($p<0.05$). After the 3-mo intervention, relatively large increases in score were observed in the "conversation" (46.0% increase, $p<0.001$), and "memory" (42.6% increase, $p<0.001$) domains, whereas that of "housework, arrangement of personal belongings" was relatively small (23.5% increase, $p<0.01$), and that of "interest, volition, social relations" (35.1% increase, $p<0.001$) was moderate. An improvement in "memory" and "conversation" appeared to be manifested in the LD+MCT group.

Post-intervention changes in cognitive functions in individual participants

To examine whether the favorable effects on cognitive function generally observed in the LD+MCT group were also observed in each individual, changes in these variables in the individual are shown in Fig. 2.

In regard to cognitive function, after the 3-mo intervention, an increase in total score for the MMSE and NM scales in the LD+MCT group was observed in 46% (6/13) and 92% (12/13) of the participants, respectively. The respective increases in the LD+LCT group were 50% (6/12) and 25% (3/12), whereas those in the control group were 9% (1/11) and 0% (0/11). Therefore, increases in the points of the NM scale were observed in most of the participants in the LD+MCT group.

Correlation between changes in cognition test scores and changes in muscle mass, strength, and function

In our previous report, we observed favorable effects on muscle strength, and function in the LD+MCT group (*4*). Therefore, it is conceivable that an increase in muscle function may lead to an increase in cognitive function, and vice versa. Pearson correlation coefficients between changes in cognition test scores and changes in muscle mass, strength, and function were examined. There was no correlation between the changes in cognition test scores and those in calculated right mid-upper-arm muscle area (AMA), calculated left AMA, right calf circumference (CC), left CC, right hand grip strength, right knee extension time, left knee extension time, walking speed, leg open and close test, or peak expiratory flow (Table 2). However, there was a significant positive correlation between the NM scale and left hand grip strength. This was due to one participant showing high increases in the scores of both tests. However, we thought that this was an exceptional case, and overall, there was no correlation between the changes in cognition test scores and those in muscle mass, strength, or function.

DISCUSSION

In the present study, we found that the daily administration of MCTs (6 g) along with a leucine- and cholecalciferol-enriched supplement with dinner for a 3-mo period substantially improved cognitive function in the frail elderly adults. The dosage in the present study amounted to a 60-fold increase in MCTs intake, a 3-fold increase in vitamin D intake, and a 30% increase in leucine intake, relative to the habitual amount of daily intakes (*4*). There were minimal increases in the intake of EAAs (14%), sodium (3%), thiamine (15%), pyridoxine (11%), and cyanocobalamin (9%) in the intervention groups. Thus, the combination of MCTs, leucine, and cholecalciferol may be a candidate supplement for improvement in cognitive function as well as muscle function as described previously (*4*). These favorable effects were not observed in the frail elderly participants who received LCTs (6 g) with the leucine- and cholecalciferol-enriched supplement, suggesting that the favorable effects that were observed in the LD+MCT group were mainly due to the administration of MCTs. An only MCT group was not set, because the primary endpoint in this study was to examine the effects of the combination of nutrients on muscle strength and function, but not those on cognitive function.

Effects of MCTs on cognitive function have been reported both acutely (with a single dose) and chronically (with multiple repeated doses) (*9*, *10*). However, the degrees of improvement of cognitive function in these studies were much smaller than that in our study. In a study examining the acute effects of MCTs, an MCT drink (40 mL) was given to participants with AD and mild cognitive impairment (mean MMSE score=22.0), and at 90 min after the MCT drink, the Alzheimer's Disease Assessment Scale-Cognitive subscale score (ADAS-cog) was determined (*9*). The ADAS-cog score was significantly improved by about −1.5 points (lower is better) only in apolipoprotein E-4 (APOE4) (−) participants ($p=0.039$). The chronic administration of 20 g/d of MCTs (3 times larger than the dose used in our study) for 90 d to participants (mean MMSE score=19.7) did not improve MMSE scores and only improved the ADAS-cog score by −1.75 points in the APOE4 (−) participants (*10*). However, because the total score of the ADAS-cog is 70 points (*11*), the improvement in the ADAS-cog score in both studies was very small. A recently conducted intervention study showed that addition of 20 g of caprylic triglycerides for 3 mo did not improve cognitive function (MMSE and ADAS-cog score) in 22 Japanese AD patients, even in those patients without the ApoE4 allele (*12*). Considering the marked increase in the scores of the NM scale and the significant increase in MMSE points in our study, better effects on cognitive function might be observed with a smaller dose of MCTs

(6 g). This is possibly due to the combination of MCTs, leucine and cholecalciferol supplementation. It should be noted that the prior studies on MCTs were performed in subjects who had been diagnosed with AD, whereas the subjects of the current study were elderly individuals. It is therefore conceivable that the subjects with definite AD did not respond to MCTs, whereas the ordinary elderly subjects showed a favorable response to MCTs.

It is unlikely that leucine supplementation per se contributes to the improvement of cognitive function because a RCT of leucine supplementation showed no such improvement (*13*). However, cholecalciferol supplementation per se may contribute to improved cognitive function. Recent meta-analyses showed that lower concentrations of vitamin D were associated with poor cognitive function and a higher risk of AD (*14*, *15*). Vitamin D supplementation may prevent cognitive decline as observed in aged rats (*16*). It is therefore possible that the combination of cholecalciferol and MCTs could have led to the more favorable effects rather than by MCTs alone.

In our previous report, we observed favorable effects on muscle strength and function in the LD+MCT group (*4*). Therefore, it is conceivable that an increase in muscle function may lead to an increase in cognitive function, and vice versa. Mostly, however, there was no correlation between the changes in cognition test scores and those in muscle mass, strength, or function. In agreement with our results, a meta-regression analysis conducted in 2006 (*17*) and subsequent RCTs (*18*, *19*) showed that no quantitative relationship could be demonstrated between the increment of fitness and the increment of cognitive performance. Thus, the relationship between physical activity and inhibition of cognitive decline might not depend on the improved fitness. Physical activity and cognitive function might be regulated not only by muscle function but also by brain volume and systemic blood flow (*20*, *21*). Therefore, it is conceivable that a mixture of MCTs, leucine, and cholecalciferol affects brain volume (or brain function) and systemic blood flow by unknown mechanisms. Future studies may clarify this issue.

Although an improvement of cognitive function in the LD+MCT group was observed in the scores of both tests, the effect size of the NM scale was much larger than that of the MMSE scale. This might be due to fundamental differences between the two tests. Indeed, correlation coefficients between changes in the MMSE and NM scales were weak and not significant ($r=0.35$, $p=0.24$). This may be because the MMSE is a questionnaire to participants that requires verbal responses, and communication by speech is sometimes difficult as dementia progresses. In addition, the MMSE evaluates the ability of the elderly participant at the place of examination, and this ability may differ from that in the participant's daily life. Therefore, behavioral assessment methods in which mental function is rated by observing behaviors in daily life might be more useful to assess behavioral changes in elderly cognitively impaired adults. In addition, we were of the opinion that the willingness and self-assertion of the participants in the LD+MCT group increased the scores on the NM scale. The NM scale could evaluate the increases in cognition, muscle function, and willingness in the LD+MCT group, while the MMSE could not. Thus, the increases in the NM scale score might be much larger than the increases in the MMSE score.

The mechanisms of MCTs-induced improvement of cognitive function have been elucidated (*22*). Medium-chain fatty acids (MCFAs) are rapidly absorbed mostly via the portal circulation and oxidized in the liver (*22*). This results in an excess of acetyl-CoA in the liver and, in turn, the rapid production of ketones, which are delivered to the brain for use as an energy source and for the synthesis of phospholipids (*23*, *24*). An increase in serum β-hydroxybutyrate (OHB) at 90 min after MCT administration correlated significantly with a better score on a cognitive test (*9*). Ketone bodies used in the brain are candidates, but it is also conceivable that MCFAs directly activate brain cells. In humans, after the first MCT meal, MCFA comprised 8% of the total chylomicron triglyceride fatty acids in plasma (*25*). At 2 h after the addition of MCTs in type 1 diabetic patients, an increase in plasma free fatty acids was observed with an improved cognitive test score (*26*). It is conceivable that in addition to the leucine and cholecalciferol, after the addition of the MCTs, blood concentrations of ketone bodies and MCFAs might be further increased and eventually improve brain function.

The present study has some limitations. Because it was conducted in a nursing home, the number of participants was small. However, statistically significant improvements in MMSE and NM scales were observed in the LD+MCT group, suggesting that the daily combined supplementation of MCTs (6 g), leucine-rich amino acids, and cholecalciferol may be a feasible diet to benefit cognition in frail elderly individuals. The 3-mo intervention period may be too short to observe the full effects on cognition, although significant favorable effects of cognition were observed within the 3-mo intervention. Although this combination of nutrients showed promise in the frail elderly participants of our study, it is not clear whether similar favorable effects would be observed by the combination of two nutrients including MCTs or in other populations.

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Author contributions

S. A., O. E., and M. S. designed the research (project conception, development of the overall research plan, and study oversight); S. A. and M. S. conducted the research (hands-on conduction of the experiments and data collection); S. A. and O. E. analyzed the data or performed the statistical analysis; O. E. wrote the manuscript; and O. E. had the primary responsibility for the final content. All of the authors read and approved the final manuscript.

Ethical standards

The authors declare that the study procedures comply with the current ethical standards for investigation involving human participants in Japan.

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